PB81-191918

Trimmed Spearman-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays

Montana State Univ.
Bozeman

Prepared for

Environmental Research Lab.
Duluth, MN

1977

U.S. Department of Commerce
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1. DATE PREPARED	2. LAB/OFFICE DRAFT NO.	3. COPYRIGHT PERMISSION (✓ one)	4. PEER REVIEW CLEARANCE (✓ one)
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5. PRESENT TITLE: Trimmed Spearman-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays

a. TECHNICAL INFORMATION PLAN TITLE AND REFERENCE

6. AUTHOR, ORGANIZATION, AND ADDRESS

Hamilton, M. A., R. C. Russo, and R. V. Thurston, Fisheries Bioassay Laboratory, Montana State University, Bozeman, Montana 59715

EPA PROJECT DOCUMENTATION

7. SERIES	8. REPORT DATE	9. CONTRACT/GRANT/IAG NUMBER
EPA-600/J-77-178	Jour. Art. April 1981	

10. TYPE OF MATERIAL (✓ one)
SPECIFY (WHERE NECESSARY)

- ☐ RESEARCH REPORT
- ☐ MEETING/PUBLICATION
- ☐ PROJECT REPORT AND PROJECT SUMMARY
- ☐ APPLICATIONS GUIDE
- ☒ JOURNAL PUBLICATION
- ☐ SUMMARY/SYNTHESIS
- ☐ UNPUBLISHED REPORT
- ☐ RESPONSE REPORT
- ☐ AUDIO VISUAL

11. PROJECT OFFICER/IN-HOUSE AUTHOR

a. SIGNATURE	b. DATE
Kenneth E. F. Hokanson	March 16, 1981

c. TYPED NAME AND ADDRESS	d. FTS TELEPHONE NO.
Kenneth E. F. Hokanson, USEPA, Monticello Ecological Research Station, Monticello, Minnesota 55362	787-3332

12. TECHNICAL INFORMATION (PROGRAM) MANAGER

a. SIGNATURE	b. DATE
for	23 March 1981

c. TYPED NAME AND ADDRESS	d. FTS TELEPHONE NO.
Evelyn P. Hunt, U.S. EPA, Environmental Research Laboratory-Duluth, 6201 Congdon Boulevard, Duluth, Minnesota 55804	783-9586

13. COMMENTS

PUBLISHED: Environ. Sci. Technol. 11(7): 714-719 (1977)

MAR 26 1981

Case file PB81 191918

EPA-350(Cin)(8-80)

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TECHNICAL REPORT DATA
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1. REPORT NO.	2.	3 PB81-191916
EPA-600/J-77-178	Journal Article	

4. TITLE AND SUBTITLE
Trimmed Spearman-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays

5. REPORT DATE
April 1981

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Hamilton, M. A., R. C. Russo, and R. V. Thurston

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Fisheries Bioassay Laboratory
Montana State University
Bozeman, Montana 59715

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.
R800861

12. SPONSORING AGENCY NAME AND ADDRESS
U.S. Environmental Protection Agency
Environmental Research Laboratory-Duluth
Monticello Ecological Research Station
Monticello, Minnesota 55362

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA-600/03

15. SUPPLEMENTARY NOTES
Environ. Sci. Technol. 11(7): 714-719 (1977)

16. ABSTRACT

Several methods for treatment of data from toxicity tests to determine the median lethal concentration (LC50) are discussed. The probit and logit models widely used for these calculations have deficiencies; therefore, a calculational method, named the "trimmed Spearman-Karber method", is developed. Examples of actual and hypothetical bioassay test data are given, and comparisons are made of the abilities of these three methods to treat these data. The trimmed Spearman-Karber method is not subject to the problems of the probit and logit models, has good statistical properties, is easy to use, and is recommended for accurate and precise calculation of LC50 values and their 95% confidence interval end points.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

PRODUCT OF:
NATIONAL TECHNICAL INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	6
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA-600/J-77-178
Journal Article

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Trimmed Spearman-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays

Martin A. Hamilton[1], Rosemarie C. Russo, and Robert V. Thurston*

Fisheries Bioassay Laboratory, Montana State University, Bozeman, Mont. 59715

■ Several methods for treatment of data from toxicity tests to determine the median lethal concentration (LC50) are discussed. The probit and logit models widely used for these calculations have deficiencies; therefore, a calculational method, named the "trimmed Spearman-Karber method", is developed. Examples of actual and hypothetical bioassay test data are given, and comparisons are made of the abilities of these three methods to treat these data. The trimmed Spearman-Karber method is not subject to the problems of the probit and logit models, has good statistical properties, is easy to use, and is recommended for accurate and precise calculation of LC50 values and their 95% confidence interval end points.

The most widely used methods for estimating the median lethal concentration (LC50) for a toxicant are based on either the integrated normal (probit) or logistic (logit) models. These models often describe the relationship between mean mortality and concentration of toxicant. Estimates of LC50 based on the probit or logit models have some deficiencies which have not previously been described to biologists. These deficiencies are sufficiently important that methods based on the probit and logit models should probably not be used for routine analyses of an extended series of bioassay experiments.

Where many sets of bioassay data are analyzed on a routine basis, it is important that all analyses be based on a single statistical method. The appropriate statistical method must have three characteristics: be reasonably accurate and precise; be programmable so that all calculations can be done on a digital computer; and be robust enough that it will not fail when the data are somewhat unusual. When a large number of bioassays are run, some anomalies are bound to occur, and the statistical method must be able to produce reasonable LC50 estimates from such anomalous data.

The following is a critical discussion of methods based on the probit and logit models and a description of a recommended alternative, named the "trimmed Spearman-Karber method".

Discussion of Probit and Logit Models and Methods for Estimating LC50

Preliminaries. Let an acute toxicity bioassay yield the following information: $x_1, x_2, \ldots, x_k$, which are the natural logarithms of the k concentrations of toxicant used and which are arranged in order of increasing concentration so that $x_1 < x_2 < \ldots < x_k$; $n_1, n_2, \ldots, n_k$, which are the numbers of fish exposed to the k concentrations, respectively; $r_1, r_2, \ldots, r_k$, which are the numbers of fish that die within a fixed time of exposure to the k concentrations, respectively.

It is not unusual in a toxicity bioassay experiment to have more than one of the low concentration tanks yield no mortalities and/or to have more than one of the high concentration tanks yield 100% mortalities. For such an experiment, let x_1 be the highest $\log_e$-concentration producing no mortality where all tanks of lower concentration also produce no mortality. Let x_k be the lowest $\log_e$-concentration producing 100% mortality where all tanks of higher concentration also produce 100% mortality. In essence, all tanks below $\log_e$-concentration x_1 and above $\log_e$-concentration x_k are not used in the analyses.

Because the fish used in the experiment were drawn at random from some large population of fish, one can talk about the population proportion of mortality at each of the k concentrations. Let $P(x)$ be the true, unknown proportion of the underlying population which would die within the fixed duration time if exposed to a $\log_e$-concentration of x. Then the observed proportion mortality $p_i = r_i/n_i$ is an estimate of

[1] Statistical Laboratory.

$P(x_i)$. Call $P(x)$ the true *response curve;* it is typically S-shaped.

One can also assume that each fish in the underlying population has a unique upper limit of tolerance to some concentration of the toxicant, which is that concentration just sufficient to kill the fish within a fixed exposure time; at any concentration less than this, a fish would live longer than the fixed exposure time. The frequency distribution of log tolerance concentrations for all fish in the population is called the tolerance distribution. Denote the *mean* of this population tolerance distribution by μ. All of the statistical methods discussed in this paper require that the population tolerance distribution is symmetric around μ. This means that μ is also the *median* of the tolerance distribution. Because the true response curve, $P(x)$, is the cumulative relative frequency curve for the tolerance distribution, $P(\mu) = \frac{1}{2}$. The symmetry of the distribution forces $P(x)$ to be symmetric in the sense that $P(\mu - \delta) = 1 - P(\mu + \delta)$, for every $\delta > 0$. Experience shows the true response curve to be nearly symmetric for acute toxicity data when x is log concentration of toxicant. The parameter of interest, LC50, is the antilog of μ. All methods described here estimate the LC50 by taking the antilog of an estimate of μ.

Probit and Logit Models. The probit model for $P(x)$ is

$$P(x) = \int_{-\infty}^{(x-\mu)/\sigma} \frac{1}{\sqrt{2\pi}} e^{-w^2/2} dw \qquad (1)$$

where σ is the standard deviation of the tolerance distribution (*1*).

The logit model for $P(x)$ is

$$P(x) = [1 + e^{-(x-\mu)/\beta}]^{-1} \qquad (2)$$

where β is a constant [0.55133] times the standard deviation of the tolerance distribution (*1*).

Equations 1 and 2 are presented to show how specific the probit and logit models are. The probit model is appropriate only if $P(x)$ is closely approximated by Equation 1; similarly, the logit model is appropriate only if $P(x)$ is closely approximated by Equation 2.

It is difficult to choose between the logit and probit models. If the true response curve is closely approximated by one model, it probably is closely approximated by the other (*2, 3*).

After one has decided that either the probit or logit model is appropriate, there are a number of statistical methods available for estimating μ. The most often-used method is the maximum likelihood procedure. An iterative technique is required (*1*). Computer programs are available to perform the calculations (*4, 5*).

The Litchfield and Wilcoxon (*6*) method, which is popular in the field of acute toxicity bioassays, is a rapid graphical method for finding the maximum likelihood estimate of μ for the probit model. The Litchfield and Wilcoxon method usually will not produce the exact maximum likelihood estimate and is not totally programmable because it requires drawing lines after visual inspection of plotted data.

The next most popular procedure for estimating μ under either the probit or logit models is the minimum transform chi-square method. The simplest version of this method amounts to fitting a single weighted least-squares linear regression line, where transformed-p is the dependent variable and x is the independent variable (*1, 3*).

Two response curve *models* and two *methods of estimation* for each model have been discussed. One should not speak of "the probit method" but should specify the method more precisely by saying, e.g., "the maximum likelihood estimate based on the probit model".

Criticism of Methods Based on Probit and Logit Models. The maximum likelihood iterative procedure can be very unstable if the data do not conform to the assumed model. Two common types of instability are that the iterations may not converge, and that the iterations may converge to different values depending on the initial guesses used to start the program. This means that if all LC50's are to be estimated by the maximum likelihood method, then some sets of potentially useful data cannot be analyzed.

Another criticism pertains to comparisons of results from duplicate experiments. Suppose that an acute toxicity bioassay is performed twice using the same toxicant concentrations and numbers of fish in each bioassay. Let the k proportional mortalities for bioassay A be $p_1^A, p_2^A, \ldots, p_k^A$ and let those for bioassay B be $p_1^B, p_2^B, \ldots, p_k^B$. Suppose that $p_i^A \leq p_i^B$, for every $i = 1, \ldots, k$, with $p_i^A < p_i^B$ for at least one concentration. Then because the mortality in bioassay B is greater than the mortality in bioassay A, one would want the LC50 estimate for B to be no larger than the LC50 estimate for A. Using either maximum likelihood or minimum transform chi-square methods, for either the probit or logit models, it can happen that the bioassay with the greater mortality yields the higher LC50 estimate.

Tables I and II show examples of these deficiencies. Table I gives five sets of actually observed experimental percentage mortality data for a control tank and five test tanks of increasing toxicant concentration. Table II gives some estimates of LC50 for the experimental data. Maximum likelihood estimates do not exist for data sets 1A and 1B. The Daum (*5*) program did not converge for data sets 1A and 1B. Surprisingly, the BMD (*4*) program did converge for these same two data sets when a good initial guess of μ was entered. The Litchfield and Wilcoxon (*6*) method failed on data sets 1D and 1E. The proportions of mortality for data sets 1B and 1C are greater than for data set 1A, but the estimated LC50's for 1B and 1C, calculated by the BMD program and the logit program, are larger than that for 1A; they are identical to that for 1A when calculated by the Litchfield and Wilcoxon method.

These examples of deficiencies are very disconcerting. It is true that the data of 1B–1E did not fit the probit and logit models. This is principally because of the unusual death of a single fish in tank 2. Even if the population tolerance distribution does correspond to the probit or logit models, it is certainly possible that an especially sensitive fish could be randomly assigned to a low concentration tank; that fish would die although no fish die in the tank of next highest concentration. The statistical methodology should not be so delicate that it is seriously affected by such a mortality.

Trimmed Spearman-Karber Method for Estimating LC50

Description of Calculations. Both the conventional and trimmed Spearman-Karber methods are model-free, requiring only the symmetry of $P(x)$. The conventional Spearman-Karber method is described in Finney (*2*). The trimming is employed in much the same way as in calculating the trimmed

Table I. Sets of Experimental Bioassay Data—Percentage Mortality

Tank	1	2	3	4	5	C
Concn, mg/L	15.54	20.47	27.92	35.98	55.52	Control
No. of test fish	20	20	20	19	20	20
Data set						
1A	0	0	0	5.26	100	0
1B	0	5	0	5.26	100	0
1C	0	5	0	15.79	100	0
1D	0	5	5	94.74	100	0
1E	0	5	5	100.00	100	0

Correction

In the article, "Trimmed Spearman-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays", by M. A. Hamilton, R. C. Russo, and R. V. Thurston [*Environ. Sci. Technol.*, 11, 714–19 (1977)], M. C. Mobley and N. R. Ferri have pointed out some errors in the calculated confidence interval endpoints given in Tables II and VI. The equation in the Appendix of our paper is correct, but we have found a programming error which resulted in most of the reported confidence intervals being too wide. For example, for data set 1C in Table II, the α%-trimmed Spearman-Karber confidence interval endpoints for $\alpha = 0, 5, 10$, and 20% should be (39.14, 44.49), (39.86, 45.38), (39.51, 46.34), and (40.77, 45.17). A listing of the FORTRAN program which we are using to do the α%-trimmed Spearman-Karber calculations is available upon request.

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Table II. Some Estimates of LC50, with Lower and Upper 95% Confidence Interval Endpoints, for Data of Table I

		Probit			Logit[d]	Spearman-Karber,[e] %			
Data set		Daum[a]	BMD[b]	L-W[c]		0	5	10	20
1A	LC50	MF[f]	39.67	40.0	42.47	43.89	44.16	44.16	44.16
	Lower	NC[g]	NC	37.4	37.37	41.73	41.75	35.98	35.98
	Upper	NC	NC	42.7	48.26	46.17	46.71	55.52	55.52
1B	LC50	MF	41.98	40.0	46.91	43.27	44.16	44.16	44.16
	Lower	NC	NC	37.4	31.22	41.35	42.09	35.98	35.98
	Upper	NC	NC	42.7	70.48	45.27	46.32	55.52	55.52
1C	LC50	40.48	40.48	40.0	44.94	41.73	42.53	42.79	42.91
	Lower	NC	NC	37.5	33.04	39.14	39.30	39.20	35.98
	Upper	NC	NC	42.7	61.14	44.49	46.02	46.70	55.52
1D	LC50	30.98	30.98	MF	30.77	31.36	31.71	31.71	31.71
	Lower	NC	NC	NC	27.00	29.74	30.65	30.70	30.70
	Upper	NC	NC	NC	35.07	33.07	32.80	32.75	32.75
1E	LC50	30.44	30.44	MF	32.37	30.80	31.48	31.48	31.48
	Lower	NC	NC	NC	25.08	29.37	27.92	27.92	27.92
	Upper	NC	NC	NC	41.77	32.30	35.98	35.98	35.98

[a] Maximum likelihood estimate based on the probit model (*5*); confidence interval using the Fieller procedure (*7*). [b] Maximum likelihood estimate based on the probit model (*4*); confidence interval end points from $\log_e(LC50) \pm 2 \times SE[\log_e(LC50)]$ (*7*). [c] Litchfield and Wilcoxon (*6*) method. The method was said to fail if 10 attempted graphs drawn did not give an acceptable line. [d] The minimum transform chi-square estimate based on the logit model. The logit transform suggested by Anscombe (*11*) was used. [e] α%-Trimmed Spearman-Karber estimates; α = 0, 5, 10, 20. [f] MF indicates that the method failed. [g] NC indicates 95% confidence interval end points are not calculable.

Table III. Example of Trimmed Spearman-Karber Calculations. Data Set 1C from Table I

Tank	(i)	1	2	3	4	5
Concn	mg/L	15.54	20.47	27.92	35.98	55.52
$\log_e$ concn	x_i	2.7434	3.0190	3.3293	3.5830	4.0167
No. of fish	n_i	20	20	20	19	20
No. of mortalities	r_i	0	1	0	3	20
Mortality proportion	p_i	0.0	0.05	0.0	0.158	1.00
Adjusted mortality proportion	$\bar{p}_i$	0.0	0.025	0.025	0.158	1.00

Table IV. Examples of Calculation of 0- and 10%-Trimmed Spearman-Karber Estimates of μ for Data of Table III

	10%		
(1) $\log_e$ of concn interval: (x_{j-1}, x_j)	(3.4724, 3.5830)	(3.5830, 3.9652)	Total
(2) Relative frequency: ${}_{10}\bar{p}_j - {}_{10}\bar{p}_{j-1}$	0.0725	0.9275	1.000
(3) Midpoint of interval: $(x_{j-1} + x_j)/2$	3.5277	3.7741	
(2) × (3)	0.25576	3.50048	3.7562

The estimate of μ is 3.7562, and the estimate of LC50 is thus $e^{3.7562}$ = 42.79 mg/L.

	0%				
(1) $\log_e$ of concn interval: (x_{j-1}, x_j)	(2.7434, 3.0190)	(3.0190, 3.3293)	3.3293, 3.5830)	(3.5830, 4.0167)	Total
(2) Relative frequency: $\bar{p}_j - \bar{p}_{j-1}$	0.025	0.0	0.133	0.842	1.000
(3) Midpoint of interval: $(x_{j-1} + x_j)/2$	2.8812	3.1742	3.4562	3.7999	
(2) × (3)	0.07203	0.0	0.45967	3.19952	3.7312

The estimate of μ is 3.7312, and the estimate of LC50 is thus $e^{3.7312}$ = 41.73 mg/L.

mean (*7*). The experimenter must choose a constant α, where $0 \leq \alpha \leq 50$, which is the percent of extreme values to be trimmed from each tail of the tolerance distribution before calculating the estimate of μ. The choice of $\alpha = 0$ produces the conventional Spearman-Karber estimate. The estimation procedure will now be described in detail using data set 1C of Table I.

The first step is to adjust $p_i, \ldots, p_k$ if these mortality proportions do not satisfy $p_1 \leq p_2 \leq \ldots \leq p_k$. This adjustment is necessary because it is known that $P(x_1) \leq P(x_2) \leq \ldots \leq P(x_k)$, and we require the p_i's to have this same monotone nondecreasing order. Thus, we will define new mortality proportions $\bar{p}_1, \bar{p}_2, \ldots, \bar{p}_k$ by combining the mortalities (r_i's) and the numbers of fish (n_i's) of any adjacent p_i's which are not in the proper monotone order to give a new averaged estimate of mortality proportion, $\bar{p}$, at the two adjacent doses. Consider Table III. Because $p_3 = 0.0$ is less than $p_2 = 0.05$, adjustment is necessary. Both $\bar{p}_2$ and $\bar{p}_3$ are set equal to $(r_2 + r_3)/(n_2 + n_3) = 1/40 = 0.025$. This process is continued until the $\bar{p}_i$'s are in a monotone nondecreasing sequence. If monotonicity is violated for more than pairs, the order in which averaging is performed is unimportant since the final $\bar{p}_i$'s will always be the same (*8*).

The second step is to plot the $(x_i, \bar{p}_i)$ points and connect them with straight lines as shown in Figure 1a. The polygonal figure formed is an estimate of $P(x)$, the cumulative relative frequency curve for the tolerance distribution.

The third step is to trim off the upper α percent and lower α percent of the polygon. Change the ordinate scale by replacing $\bar{p}$ with ${}_{\alpha}\bar{p} = (\bar{p} - \alpha/100)/(1 - 2\alpha/100)$. Ignore those ${}_{\alpha}\bar{p}$values which are less than zero or greater than one. The resulting polygon is an estimate of the cumulative relative frequency curve for the central $(100 - 2\alpha)$ percent of the tolerance distribution. If the experimenter does not want to

trim, then $\alpha = 0$, and this third step does not alter the polygon formed in step 2.

The trimming procedure for the example is shown in Figures 1a and 1b for $\alpha = 10$. By linear interpolation one finds that in Figure 1a, $\bar{p} = 0.10$ (or equivalently, ${}_{10}\bar{p} = 0.0$) corresponds to $x = 3.4724$ and $\bar{p} = 0.90$ (or equivalently, ${}_{10}\bar{p} = 1.0$) corresponds to $x = 3.9652$. Figure 1b is an estimate of the cumulative relative frequency curve for the central 80% of the population tolerance distribution; it is the end product of the trimming procedure.

The fourth and final step is to calculate the mean associated with the cumulative relative frequency polygon formed in step 3. This mean is the α%-trimmed Spearman-Karber estimate of μ. The procedure for calculating this mean for $\alpha = 10$ is illustrated in Table IV for the polynomial of Figure 1b. The $\log_e$-concentrations $x_1, \ldots, x_k$ form $k - 1$ adjacent intervals $(x_1, x_2), (x_2, x_3), \ldots, (x_{k-1}, x_k)$. The estimated proportion of population tolerance $\log_e$-concentrations which are in the interval (x_{j-1}, x_j) is ${}_{10}\bar{p}_j - {}_{10}\bar{p}_{j-1}$, where $j = 2, 3, \ldots, k$, and where ${}_{10}\bar{p}_j$ is the proportion on the polygon corresponding to x_j. Each product of the proportion ${}_{10}\bar{p}_j - {}_{10}\bar{p}_{j-1}$ times the interval midpoint $(x_{j-1} + x_j)/2$ is found, and the sum of these products is the mean associated with the polygon. This mean is the 10%-trimmed Spearman-Karber estimate of μ for the data of Table III; the LC50 estimate is then the antilog of the estimate of μ.

Consider the data of Table III and Figure 1a; let $\alpha = 0$. Then the frequency distribution and sample mean which one would associate with the polygon are calculated as shown in Table IV. Notice that neither the 0%-trimmed (the conventional Spearman-Karber estimate) nor the 10%-trimmed Spearman-Karber estimates are greatly affected by the anomalous response in tank 2.

The above description is appropriate if no mortalities occurred in the control tank. If mortalities did occur in the control tank, one should adjust the p_i's by Abbott's formula (*1*) before starting the calculations.

Properties of Trimmed Spearman-Karber Estimator. The trimmed Spearman-Karber estimator does have the necessary three characteristics previously stated. The calculations described can be programmed for a computer and/or can be done on a desk calculator. If $\alpha \geq 100\,\bar{p}_1$ and $\alpha \geq 100(1 - \bar{p}_k)$, the method *never fails*, no matter which unusual mortality pattern is observed. (One can revise the method to calculate the α^*%-trimmed Spearman-Karber estimate, where α^* is the maximum of the three values: α, $100\,\bar{p}_1$, $100(1 - \bar{p}_k)$. This revised method always provides an estimate of μ if $\bar{p}_1 \leq 0.5 \leq \bar{p}_k$.) In duplicate experiments, if experiment B has greater mortality proportions than experiment A, then the trimmed Spearman-Karber estimate of μ for B will *never* be greater than the estimate for A as long as $\alpha \geq 100\,\bar{p}_1$ and $\alpha \geq 100(1 - \bar{p}_k)$ in both bioassays.

Regarding the accuracy and precision of the estimator, the good statistical properties of the conventional Spearman-Karber estimator are well known (*2, 8, 9*). The trimmed version of the Spearman-Karber estimator has equally good properties, but is not as sensitive to anomalous responses as is the conventional version. Equations for calculating an approximate standard error to attach to the trimmed Spearman-Karber estimator are given in the Appendix.

Because the α%-trimmed Spearman-Karber estimation procedure does not fail when $\alpha \geq 100\,\bar{p}_1$ and $\alpha \geq 100(1 - \bar{p}_k)$ and because the sensitivity of the procedure to anomalous responses decreases as α is increased, it appears that one should choose α as large as feasible. However, the standard error of the estimate increases as α increases. The choice of α then is a matter of judgment. For a group of experiments where the lowest concentrations cause approximately 5% mortality or less, and/or the highest concentrations cause approximately 95% mortality or more, we recommend a choice of $\alpha = 10$.

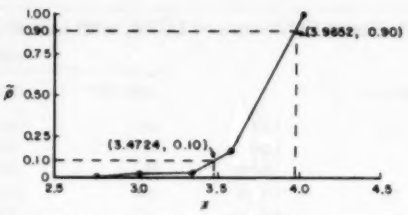

Figure 1a. Estimated cumulative relative frequency polygon for adjusted data from Table III

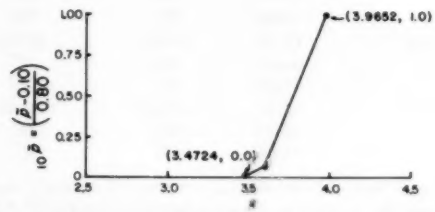

Figure 1b. Estimated cumulative relative frequency polygon of Figure 1a after trimming 10% from each tail

Table V. Hypothetical Sets of Data and Acceptable Results (*10*)—Percentage Mortality

Tank	1	2	3	4	5	6	C
Concn, µg/L	7.8	13	22	36	60	100	Control
No. of test fish	10	10	10	10	10	10	10
Data set							
4A	0	0	10	100	100	100	0
4B	0	0	70	100	100	100	0
4C	0	0	10	40	100	100	0
4D	0	0	20	70	100	100	0
4E	0	0	20	30	100	100	0

Performance of Estimators on Test Data

The Committee on Methods for Toxicity Tests with Aquatic Organisms (*10*) has published hypothetical test data and "acceptable ranges" for the associated LC50 estimates and their 95% confidence limits (Table V) to help scientists evaluate estimation procedures. Their report does not contain the criteria by which the "acceptable ranges" were determined. These data have been analyzed by some of the methods mentioned in this paper, and results are shown in Table VI. The Daum (*5*) probit program did not converge for data sets 4A and 4B; the fact that the estimates produced are acceptable is fortuitous. The BMD (*4*) probit program did converge but gave negative variance estimates for data sets 4A and 4B. For data set 4A the estimate based on the probit model is not in the acceptable range. For data sets 4C and 4E the estimate based on the probit model equals the lowest acceptable value. For data set 4D the estimate based on the logit model equals the lowest acceptable value. For data set 4C the 10- and 20%-trimmed Spearman-Karber estimates are larger than the highest acceptable value. We believe that for data set 4C, an estimate as large as 42.4, which is the value found by linear extrapolation from the responses in tanks 3 and 4, is more reasonable than an estimate smaller than 36.0, where the response was 40% mortality. Notice that some of the 95% confidence intervals of all three methods (probit, logit, and Spearman-Karber) are wider than deemed acceptable. In our

Table VI. Results Calculated by Different Methods for Data of Table V

Data set		Acceptable values	Probit		Logit[c]	Spearman-Karber,[d] %			
			Daum[a]	BMD[b]		0	5	10	20
4A	LC50	25.5–27.5	26.4[e]	24.1[f]	26.2	26.7	27.2	27.4	27.4
	Lower[g]	21.1–24.0	NC[h]	NC	20.3	22.6	22.5	22.0	22.0
	Upper[g]	28.6–30.8	NC	NC	33.6	31.7	32.8	36.0	36.0
4B	LC50	19.0–21.5	20.0[e]	21.0	20.0	19.7	19.5	19.4	19.1
	Lower	14.7–19.4	NC	NC	16.6	15.2	14.7	14.3	13.8
	Upper	22.9–24.5	NC	NC	24.1	25.5	25.9	26.2	26.4
4C	LC50	35.5–37.2	35.5	35.5	36.4	36.1	37.0	37.5[f]	38.2[f]
	Lower	26.1–30.7	28.8	26.1	27.7	28.7	29.0	29.3	28.1
	Upper	43.4–45.3	44.1	48.4	47.8	45.5	47.2	48.0	51.8
4D	LC50	29.4–30.0	29.5	29.5	29.4	29.5	29.7	29.8	29.7
	Lower	23.5–23.9	23.8	24.3	23.8	23.2	22.8	22.7	23.3
	Upper	36.3–37.4	36.6	35.8	36.5	37.7	38.6	39.1	38.0
4E	LC50	35.4–40.5	35.4	35.4	37.5	36.1	37.1	38.1	40.2
	Lower	28.1–30.8	28.2	26.2	26.5	28.3	28.4	28.7	29.6
	Upper	44.5–46.0	44.5	47.7	52.9	46.1	48.3	50.5	54.7

[a] Maximum likelihood estimate based on the probit model (*5*); confidence interval using the Fieller procedure (*1*). [b] Maximum likelihood estimate based on the probit model (*4*); confidence interval end points from $\log_e(\text{LC50}) \pm 2 \times \text{SE}[\log_e(\text{LC50})]$ (*1*). [c] The minimum transform chi-square estimate based on the logit model. The logit transform suggested by Anscombe (*11*) was used. [d] α%-Trimmed Spearman-Karber estimates; α = 0, 5, 10, 20. [e] Probit method did not converge. [f] Indicates point estimates of LC50 which are not in the range of acceptable values. [g] Lower and upper 95% confidence interval end points. [h] NC indicates 95% confidence interval end points are not calculable.

opinion, however, the intervals are narrow enough to be of practical value.

Conclusions

The α%-trimmed Spearman-Karber calculations have been programmed in FORTRAN and are performed routinely in our laboratory by use of the Montana State University XDS Sigma 7 computer. The program is integrated with a plotting routine for obtaining plots of toxicity curves, LC50 vs. time. We have analyzed hundreds of bioassay data sets using the α%-trimmed Spearman-Karber method, the maximum likelihood method for the probit model, and the minimum transform chi-square method for the logit model. The α%-trimmed Spearman-Karber method always provided useful results, but the methods based on the logit and probit models exhibited all of the deficiencies previously described.

Based on our experience with real and hypothetical data and taking into account accuracy, precision, computability, and robustness, we conclude that the α%-trimmed Spearman-Karber procedure is overall a better method than the methods based on the probit and logit models.

Appendix

Variance Formulas. Let $x_1, \ldots, x_k$ and $n_1, \ldots, n_k$ be as defined in the Preliminaries section. Let $\bar{p}_i, \ldots, \bar{p}_k$ be as defined in the description of the first step in calculating the α%-trimmed Spearman-Karber estimate. Let $A = \alpha/100$; $L = \max\{i: \bar{p}_i \leq A\}$; and $U = \min\{i: \bar{p}_i \geq 1 - A\}$. Then x_L is the largest $\log_e$-concentration for which the adjusted proportion response is less than or equal to A, and x_U is the smallest $\log_e$-concentration for which the adjusted proportion response is greater than or equal to $1 - A$.

Define:

$$V_1 = [(x_{L+1} - x_L)(\bar{p}_{L+1} - A)^2/(\bar{p}_{L+1} - \bar{p}_L)^2]^2 \times \bar{p}_L(1 - \bar{p}_L)/n_L$$

$$V_2 = [(x_L - x_{L+2}) + (x_{L+1} - x_L)(A - \bar{p}_L)^2/(\bar{p}_{L+1} - \bar{p}_L)^2]^2 \times \bar{p}_{L+1}(1 - \bar{p}_{L+1})/n_{L+1}$$

$$V_3 = \sum_{i=L+2}^{U-2} (x_{i-1} - x_{i+1})^2 \bar{p}_i(1 - \bar{p}_i)/n_i$$

$$V_4 = [(x_{U-2} - x_U) + (x_U - x_{U-1})(\bar{p}_U - 1 + A)^2/(\bar{p}_U - \bar{p}_{U-1})^2]^2 \bar{p}_{U-1}(1 - \bar{p}_{U-1})/n_{U-1}$$

$$V_5 = [(x_U - x_{U-1})(1 - A - \bar{p}_{U-1})^2/(\bar{p}_U - \bar{p}_{U-1})^2]^2 \times \bar{p}_U(1 - \bar{p}_U)/n_U$$

$$V_6 = \{[(x_U - x_{L+1})(1 - A - \bar{p}_U)^2/(\bar{p}_U - \bar{p}_{L+1})^2] - [(x_{L+1} - x_L)(A - \bar{p}_L)^2/(\bar{p}_{L+1} - \bar{p}_L)^2] + (x_L - x_U)]^2 \bar{p}_{L+1}(1 - \bar{p}_{L+1})/n_{L+1}$$

Let $\hat{\mu}$ denote the α%-trimmed Spearman-Karber estimate and $\hat{\text{Var}}(\hat{\mu})$ denote the estimated variance of $\hat{\mu}$. We are at present using these formulas, which are first order approximations, for $\hat{\text{Var}}(\hat{\mu})$:

For $U - L \geq 4$,

$$\hat{\text{Var}}(\hat{\mu}) = (V_1 + V_2 + V_3 + V_4 + V_5)/(2 - 4A)^2$$

For $U - L = 3$,

$$\hat{\text{Var}}(\hat{\mu}) = (V_1 + V_2 + V_4 + V_5)/(2 - 4A)^2$$

For $U - L = 2$,

$$\hat{\text{Var}}(\hat{\mu}) = (V_1 + V_5 + V_6)/(2 - 4A)^2$$

For $U - L = 1$,

$$\hat{\text{Var}}(\hat{\mu}) = (x_U - x_L)^2[[(0.5 - \bar{p}_U)^2/(\bar{p}_U - \bar{p}_L)^4]\bar{p}_L(1 - \bar{p})_L/n_L + [(0.5 - \bar{p}_L)^2/(\bar{p}_U - \bar{p}_L)^4]\bar{p}_U(1 - \bar{p}_U)/n_U]$$

We are calculating approximate 95% confidence interval end points for μ using

$$\hat{\mu} \pm 2.0\,[\hat{\text{Var}}(\hat{\mu})]^{1/2}$$

We have calculated $\hat{\text{Var}}(\hat{\mu})$ for more than 32 000 simulated data sets. These calculations indicate that if $U - L$ is greater than 1, $\hat{\text{Var}}(\hat{\mu})$ is a conservative estimate of the variance. Consequently, the confidence intervals are wider than necessary to provide 95% confidence.

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Received for review August 12, 1976. Accepted March 8, 1977. Research funded in part by the U.S. Environmental Protection Agency, Duluth, Minn., Research Grant Nos. R800861 and R803950.